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EXAMINER	
BERTAGNA, ANGELA MARIE	

ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/600,201	SLEPNEV, VLADIMIR I.	
	Examiner	Art Unit	
	Angela Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 April 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-75* is/are pending in the application.

4a) Of the above claim(s) *74 and 75* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-73* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *20 June 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *10/8/03; 8/16/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election of Group I, claims 1-73, in the reply filed on April 17, 2006 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 74 and 75 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on April 17, 2006.

Claim Rejections - 35 USC § 102

2. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43 are rejected under 35 U.S.C. 102(b) as being anticipated by Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited in IDS).

Regarding claim 1, Myakishev teaches a method of determining for a given nucleic acid sample, the identity of the nucleotide at a known polymorphic site (see Figure 2 and Results section, pages 163-165), said method comprising:

a) subjecting to an amplification regimen a population of primer extension products generated from a nucleic acid sample (Figure 2, top panel, where extension with the tailed primers occurs in round 1 of the amplification; also page 164, col. 2), each primer extension product comprising a tag sequence (the tail), which tag sequence specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, column 1, teaches that the each allele-specific primer contains a different 21-base tail at the 5'end), wherein said amplification regimen is performed using an upstream amplification primer (the "reverse primer" of page 163, col. 2 and page 164, col. 2) and a set of distinguishably labeled downstream amplification primers (the two energy-transfer (ET) primers of Fig. 2, see also page 164, col. 2), each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products and a distinguishable label (see Fig. 1 for the structure of the ET primers), wherein each distinguishable label specifically corresponds to the presence of a specific nucleotide at said polymorphic site (Fig. 2 teaches red and green labels for different mutations)

b) detecting incorporation of a distinguishable label into a nucleic acid molecule, thereby to determine the identity of the nucleotide at said polymorphic site (see Fig. 2 and pages 164-165).

Regarding claim 2, the distinguishable label is fluorescent (see Fig. 2 and page 164).

Regarding claim 5, the amplification reaction comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers (see Figure 2 and page 164; see also Methods section, page 167-168 for the reaction conditions).

Regarding claim 10, the tag sequence is 21 nucleotides (page 164, column 1).

Regarding claim 12, Myakishev teaches a pair of fluorescently-labeled downstream amplification primers each including a tag added in the first round of extension and corresponding to the presence of a first and second allele, respectively, at the polymorphic site (Figure 1 teaches the structure of the ET primers; see also page 164, col. 2).

Regarding claim 18, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Fig. 2 and pages 163-165; note that 9 SNPs were tested), said method comprising:

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample (Figure 2, top panel, where extension with the tailed primers occurs in round 1 of the amplification; also page 164, col. 2), each primer extension product comprising a member of a set of tag sequences (the tail added in step 1), which tag sequence specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, col. 1 teaches that the allele-specific primers contain different 5' tags), wherein said amplification regimen is performed using one upstream amplification primer for each sequence comprising a known polymorphic site to be interrogated (the reverse primer, page 163), and a set of distinguishably labeled downstream amplification primers (the ET primers; Fig. 2), each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products (see Figure 1) and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site (see Figures 1 & 2), and wherein said upstream amplification

primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product (see page 164, col. 1, where the upstream primer was hybridized at different points on the template to produce differently sized amplicons)

b) detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at each said polymorphic site (see Fig. 2 and also the Methods section, page 168, where fluorescence is detected).

Regarding claim 19, the label is fluorescent (see Fig. 2).

Regarding claim 22, the amplification reaction comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers (see Figure 2 and page 164; see also Methods section, page 167-168 for the reaction conditions).

Regarding claim 27, Myakishev teaches that the tag sequence is 21 nucleotides (page 164, col. 1).

Regarding claim 34, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Fig. 2 and pages 163-165; note that 9 SNPs were tested), said method comprising:

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample (Figure 2 and page 164, col. 2), each primer extension product comprising a first tag sequence (generated by extension of the reverse primer) or its complement and a member of a set of second tag sequences or its complement (the 21 nt tail

added in step 1 of the reaction), the presence of which second tag sequence or its complement specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, col. 1), wherein for each polymorphic site in said set of polymorphic sites, said first tag sequence is located at a distinct distance 5' of said polymorphic site, relative to the distance of said first tag sequence from a polymorphic site on molecules in said sample containing other polymorphic sites (page 164, col. 1 teaches that the reverse primer may hybridize at different positions on the template), wherein said amplification regimen is performed using an upstream amplification primer comprising said first tag sequence (the reverse primer, page 163), and a set of distinguishably labeled downstream amplification primers (the ET primers), each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products (the tail sequence; see Fig. 1) and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site (see Fig. 1 and 2), and wherein said upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product (page 164, col. 1 teaches hybridization of the reverse primer at different locations on the template)

b) detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at each said polymorphic site (page 168, col. 1).

Regarding claim 35, Myakishev teaches fluorescent labels (Fig. 2).

Regarding claim 38, the amplification reaction comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2)

oligonucleotide primer annealing; and 3) polymerase extension of annealed primers (see Figure 2 and page 164; see also Methods section, page 167-168 for the reaction conditions).

Regarding claim 43, the tag is 21 nucleotides in length (page 164, col. 1).

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. Claims 3-4, 9, 20-21, 26, 36, 37, and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited in IDS) in view of Piggee et al. (J Chromatogr A. 1997 Sep 26; 781(1-2): 367-75; cited in IDS).

Myakishev teaches the method of claims 1, 18, and 34, as discussed above.

Myakishev teaches fluorescence detection rather than capillary electrophoresis.

Regarding claims 9, 26, and 42, Myakishev teaches a thermal cycling device with a fluorescence detection system (Methods, page 168). Note that the specification does not require that the elements of claims 9, 26, and 42 (a thermal cycling device, a sampling device, a capillary electrophoresis device and a fluorescence detector) be physically connected.

Regarding claims 3-4, 20-21, 36, and 37, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see abstract).

Regarding claims 9, 26, and 42, Piggee teaches a capillary electrophoresis device with a sampling device, and a fluorescence detection system (pages 368-369).

Piggee teaches that this method is fast, avoids radioactive labels, and has a high automation potential. Although Piggee used capillary electrophoresis to detect point mutations analyzed by single base extension reactions, it is expressly stated that the method would also be applicable to other methods of mutation detection including competitive priming with end mismatch, etc. Piggee also noted that the capillary electrophoresis method facilitated multiplexing through the use of different length primers (see Conclusion section, page 375).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to utilize capillary electrophoresis as an additional or alternative method of detection in the mutation detection method of Myakishev. Piggee expressly taught the advantages of a capillary electrophoresis detection system, namely, rapid analysis with a high potential for automation and multiplexing (see above). The ordinary practitioner would have been motivated by the teachings of Piggee to additionally (or alternatively) analyze the SNP detection results using capillary electrophoresis in order to more rapidly and automatically screen a larger number of samples, thus resulting in the instantly claimed methods.

5. Claims 6-8, 23-25, and 39-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited in IDS) in view of Wiesner et al. (Biochemical and Biophysical Research Communications (1992) 183(2): 553-559) and further in view of Piggee et al. (J Chromatogr A. 1997 Sep 26; 781(1-2): 367-75; cited in IDS).

Myakishev teaches the method of claims 1-2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43, as discussed above.

Myakishev does not teach analyzing an aliquot of the PCR during the reaction process. Also, Myakishev teaches fluorescence detection rather than capillary electrophoresis.

Wiesner teaches a method of determining the initial concentration of a PCR template by measuring the amount of product accumulating in consecutive cycles (see abstract).

Regarding claims 6-7, 23-24, 39, and 40, Wiesner teaches taking an aliquot after each cycle of a PCR reaction and analyzing the amount of accumulating product by agarose gel electrophoresis and scintillation counting (page 554, Methods section). Wiesner teaches that by analyzing aliquots the need for standardization of the signal due to differing initial product concentrations is eliminated. The teachings of Wiesner also permit optimization of cycling parameters and an early identification of the reaction product (page 555, where the product is detectable halfway through the amplification reaction).

Wiesner does not teach analysis using capillary electrophoresis.

Regarding claims 8, 25, and 41, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see abstract).

Piggee teaches that this method is fast, avoids radioactive labels, and has a high automation potential. Although Piggee used capillary electrophoresis to detect point mutations analyzed by single base extension reactions, it is expressly stated that the method would also be applicable to other methods of mutation detection including competitive priming with end mismatch, etc. Piggee also noted that the capillary electrophoresis method facilitated multiplexing through the use of different length primers (see Conclusion section, page 375).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to incorporate the teachings of Wiesner and Piggee into the genotyping method of

Myakishev. Wiesner particularly pointed out the advantages of taking an aliquot after each cycle of the amplification reaction, namely: the ability to rapidly optimize the reaction conditions, to quickly determine the identity and quantity of the product, and also determine the initial concentration of the template for standardization purposes (discussed above). The ordinary practitioner would have been motivated by the teachings of Wiesner to analyze an aliquot of the PCR reaction of Myakishev in order to incorporate the above advantages into the method. Furthermore, the ordinary practitioner would have been motivated by the teachings of Piggee to substitute capillary electrophoresis for the agarose electrophoresis and scintillation counting taught by Wiesner. As discussed above, Piggee taught that capillary electrophoresis offered a rapid, radiation-free, automation-friendly, and high-throughput method for analyzing DNA samples (see above). The ordinary artisan, interested in combining the teachings of Myakishev and Wiesner in order to more rapidly detect mutations, would have been further motivated to use capillary electrophoresis, as suggested by Piggee, in order to further increase the speed, multiplexing ability, automation and throughput of the assay. Therefore, the combined teachings of Myakishev, Wiesner, and Piggee result in the instant claims 6-8, 23-25, and 39-41.

6. Claims 11, 13-17, 28-33, 44-52, 54, 58-64, 66, and 70-73 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited in IDS) in view of Nolan et al. (USPN 6,287,766 B1).

Myakishev teaches the method of claims 1-2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43, as discussed above.

Regarding claims 50 and 62, Mytakishev teaches a method of determining the identity of a single nucleotide at a known polymorphic site (see Fig. 2, and pages 163-165), said method comprising:

I) providing a nucleic acid sample comprising said polymorphic site (Fig. 2)

II) separating the strands of said nucleic acid sample and re-annealing in the presence of:

a) a first oligonucleotide primer comprising a 3' region that hybridizes to a sequence at a known distance downstream of said known polymorphic site, said first oligonucleotide primer comprising a first sequence tag located 5' of said 3' region (the reverse primer of Fig. 2; note that since the specification defines a tag as any nucleic acid sequence in a primer, any dinucleotide of the reverse primer is a tag)

b) a set of second oligonucleotide primers (the allele-specific primers of Fig. 2), wherein each member of said set comprises:

i) a region that hybridizes 5' of and adjacent to said polymorphic site

ii) a variable 3' terminal nucleotide, wherein, when said member is hybridized to said known sequence, said 3' terminal nucleotide is opposite said polymorphic site, and wherein, if and only if said 3' terminal nucleotide is complementary to the nucleotide at said polymorphic site, said 3' terminal nucleotide base pairs with said nucleotide at said polymorphic site (see Fig. 2 and page 164)

iii) a tag sequence that corresponds to said variable 3'-terminal nucleotide of (ii), said tag sequence located 5' of the region of (i) on said member (see the tail sequence in Fig. 2)

III) contacting the annealed oligonucleotides resulting from step (II) with a nucleic acid polymerase under conditions that permit the extension of an annealed oligonucleotide such that extension products are generated, wherein the primer extension product from the first oligonucleotide primer, when separated from its complement, can serve as a template for the synthesis of the extension product of a member of the set of second oligonucleotide primers (see Fig. 2), and vice versa

IV) repeating strand separating and contacting steps (II) and (III) two times, such that a population of nucleic acid molecules is generated that comprises both a sequence identical to or complementary to said first oligonucleotide and a sequence identical to or complementary to one of the members of said second set of oligonucleotides (see Fig. 2 and page 164)

V) subjecting said population of nucleic acid molecules to an amplification regimen, wherein said amplification regimen is performed using an upstream amplification primer comprising the first sequence tag comprised by said first oligonucleotide primer (the reverse primer, as discussed above), and a set of downstream amplification primers, each member of said set of downstream amplification primers comprising a tag comprised by a member of said set of second oligonucleotide primers and a distinguishable label (the energy transfer primers that comprise the tail sequence; see Fig. 1)

VIII) detecting incorporation of at least one distinguishable label, thereby determining the identity of the nucleotide at said known polymorphic site (see Fig. 2 and page 168).

Note that the designations "upstream" and "downstream" are arbitrary designations, and that the primers of Myakishev are simply reversed in terms of polarity compared to the instant claim.

Regarding claims 50 and 62, Myakishev does not teach exonuclease digestion. Also, regarding claim 62, Myakishev does not teach the use of multiple first primers.

Regarding claims 51 and 63, Myakishev teaches fluorescent labels (Fig. 2).

Regarding claims 54 and 66, the amplification reaction comprises at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers (see Figure 2 and page 164; see also Methods section, page 167-168 for the reaction conditions).

Regarding claims 58 and 70, Myakishev teaches that the tail portions of the allele-specific primers are 21 nucleotides in length (page 164, col. 1). Also, since the entire 20 nt reverse primer comprises a tag, both the first and second tags comprise 15 to 40 nucleotides.

Regarding claims 59, 60, 71, and 72, the 20 nt reverse primer hybridizes with perfect complementarity to the target downstream of the polymorphic site, and the allele-specific primers have a 22 nt target-specific portion that hybridizes upstream of the polymorphic site (see Table 1).

Regarding claims 52 and 64, Myakishev does not teach separating the nucleic acid molecules by size and/or charge.

Regarding claims 61 and 73, Myakishev teaches using a set of downstream primers comprising two different nucleotide bases at the 3'end, for example, A and G (see Figure 2), but does not teach using a set of downstream primers consisting of four primers, each with a different 3'-terminal nucleotide.

Nolan teaches a method of identifying polymorphisms using flow cytometry.

Regarding claims 50 and 62, Nolan teaches an embodiment wherein unincorporated primers from an initial amplification reaction may be degraded using the heat labile Exonuclease I followed by polymerase extension, where the initial denaturation step destroys the activity of the exonuclease (see for example, column 5, line 60 – column 6, line 20). Nolan also teaches multiplex detection of mutations using multiple sets of primers (column 7, lines 60-63).

Regarding claims 52 and 64, Nolan teaches separation of the amplified products by flow cytometry (column 7, lines 60-63).

Regarding claims 61 and 73, the method taught by Nolan in Example 5 (column 7, lines 1-63) comprises an oligonucleotide ligation assay (OLA) followed by PCR amplification with an upstream primer and a set of downstream primers that may be fluorescently labeled (column 7, lines 11-50). Nolan teaches that the downstream primers each have a different 3'terminal nucleotide (column 7, lines 27-30) in order to identify the polymorphism in a single reaction.

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to incorporate the teachings of Nolan into the SNP genotyping method of Myakishev. The person of ordinary skill would have recognized that not all polymorphic sites may be genotyped using only two allele-specific downstream primers, and therefore, would have been motivated by the teachings of Nolan to utilize a subset of four downstream primers each with a different 3'terminus, in order to accurately type these polymorphisms. Also, the ordinary practitioner would have been motivated by the teachings of Nolan to perform a multiplexed analysis, utilizing multiple primer pairs in order to more rapidly and reproducibly genotype

several sites simultaneously. Furthermore, the person of ordinary skill would have been motivated by Nolan to incorporate an exonuclease digestion step following the initial primer extension reaction with the allele-specific primers. Although Myakishev attempted to avoid mispriming by careful design of the allele-specific and energy-transfer primers (see page 164, col. 1), incorporation of an exonuclease digestion step following the initial extension would have eliminated the possibility of such mispriming events, and thereby improved the accuracy of the method. Therefore, the person of ordinary skill, interested in obtaining a method to more rapidly and accurately determine the identity of multiple polymorphic sites using the method of Myakishev, would have been motivated to use multiple primer sets where the downstream primer sets contain all possible 3'terminal nucleotides and also to incorporate an exonuclease digestion step, thus resulting in the instantly claimed methods.

7. Claims 53, 57, 65, and 69 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited in IDS) in view of Nolan et al. (USPN 6,287,766 B1) and further in view of Piggee et al. (J Chromatogr A. 1997 Sep 26; 781(1-2): 367-75; cited in IDS).

The combined teachings of Myakishev and Nolan result in the instant claims 50, 62, and 64, as discussed above.

Regarding claims 57 and 69, Myakishev teaches a thermal cycling device with a fluorescence detection system (Methods, page 168). Note that the specification does not require that the elements of claims 57 and 69 (a thermal cycling device, a sampling device, a capillary electrophoresis device and a fluorescence detector) be physically connected.

Neither Myakishev nor Nolan teaches the use of capillary electrophoresis.

Regarding claims 53 and 65, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see abstract).

Regarding claims 57 and 69, Piggee teaches a capillary electrophoresis device with a sampling device, and a fluorescence detection system (pages 368-369).

Piggee teaches that this method is fast, avoids radioactive labels, and has a high automation potential. Although Piggee used capillary electrophoresis to detect point mutations analyzed by single base extension reactions, it is expressly stated that the method would also be applicable to other methods of mutation detection including competitive priming with end mismatch, etc. Piggee also noted that the capillary electrophoresis method facilitated multiplexing through the use of different length primers (see Conclusion section, page 375).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to utilize capillary electrophoresis as an additional or alternative method of detection in the mutation detection method resulting from the combined teachings of Myakishev and Nolan. Piggee expressly taught the advantages of a capillary electrophoresis detection system, namely, rapid analysis with a high potential for automation and multiplexing (see above). The ordinary practitioner would have been motivated by the teachings of Piggee to additionally (or alternatively) analyze the SNP detection results using capillary electrophoresis in order to more rapidly and automatically screen a larger number of samples, thus resulting in the instantly claimed methods.

8. Claims 55-56, 67, and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited in IDS) in view of Nolan et al. (USPN 6,287,766 B1) and further in view of Wiesner et al. (Biochemical and Biophysical Research Communications (1992) 183(2): 553-559).

The combined teachings of Myakishev and Nolan result in the instant claims 54 and 66, as discussed above.

Neither Myakishev nor Nolan teaches analyzing an aliquot of the PCR during the reaction process.

Wiesner teaches a method of determining the initial concentration of a PCR template by measuring the amount of product accumulating in consecutive cycles (see abstract).

Regarding claims 55, 56, 67, and 68, Wiesner teaches taking an aliquot after each cycle of a PCR reaction and analyzing the amount of accumulating product by agarose gel electrophoresis and scintillation counting (page 554, Methods section). Wiesner teaches that by analyzing aliquots the need for standardization of the signal due to differing initial product concentrations is omitted. The teachings of Wiesner also permit optimization of cycling parameters and an early identification of the reaction product (page 555, where the product is detectable halfway through the amplification reaction).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to incorporate the teachings of Wiesner into the genotyping method resulting from the combined teachings of Myakishev and Nolan. Wiesner particularly pointed out the advantages of taking an aliquot after each cycle of the amplification reaction, namely: the ability to rapidly optimize the reaction conditions, to quickly determine the identity and quantity of the product,

and also determine the initial concentration of the template for standardization purposes (discussed above). The ordinary practitioner would have been motivated by the teachings of Wiesner to analyze an aliquot of the PCR reaction generated in the method of Myakishev and Nolan in order to incorporate the above advantages into the method. Therefore, the ordinary artisan, interested in more rapidly detecting mutations, would have been motivated to take an aliquot of the amplification reaction for analysis, as suggested by Wiesner, thus resulting in the methods of the instant claims 55, 56, 67, and 68.

Conclusion

9. No claims are currently allowable.

The prior art made of record but not relied upon is considered relevant to applicant's disclosure: Germer et al (Genome Research (1999) 9:72-78) teach a method of genotyping SNPs using two allele-specific primers, one with a GC-rich tail.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela Bertagna whose telephone number is (571) 272-8291. The examiner can normally be reached on M-F 7:30-5 pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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